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Capsule

In-situ incorporation of Thomas Basic Slag into a landfilled metal-contaminated sediment decreases metal mobility and ecotoxicity and increases bacterial activity.

Abstract

Metal immobilization may contribute to the environmental management strategy of dredged sediment landfill sites contaminated by metals. In a field experiment, amendment effects and efficiency were investigated, focusing on plants, springtails and bacteria colonisation, metal extractability and sediment ecotoxicity. Conversely to hydroxylapatite (HA, 3 % DW), the addition of Thomas Basic Slag (TBS, 5 % DW) to a 5-yr deposited sediment contaminated with Zn, Cd, Cu, Pb and As resulted in a decrease in the 0.01M $\text{Ca}(\text{NO}_3)_2$ extractable concentrations of Cd and Zn. Shoot Cd and Zn concentration in *Calamagrostis epigejos*, the dominant plant species, also decreased in the presence of TBS. The addition of TBS and HA reduced sediment ecotoxicity and improved the growth of the total bacterial population. Hydroxylapatite improved plant species richness and diversity and decreased antioxidant enzymes in *C. Epigejos* and *Urtica dioica*. Collembolan communities did not differ in abundance and diversity between the different treatments.

Keywords : basic slag; *Calamagrostis epigejos*; dredged sediment; hydroxylapatite; ecotoxicity

1. Introduction

Human activities during the last decades have contaminated canal sediments with various organic and inorganic pollutants. Those of most concern are metal(loid)s, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and mineral oils. Polluted sediments in canals may be disposed on land. Indeed, the maintenance of waterways requires dredging on a regular basis to prevent flooding, facilitate navigation, and allow for use of a given water system. European Community policy encourages sediment recovery (Directive 2008/98/EC). Nevertheless, due to the high concentration of pollutants and their potential toxicity, contaminated dredged sediments cannot be used in civil engineering as raw material or the deposit cannot be used to produce valuable biomass. Many treatments are available for contaminated sediments, but relatively few are applicable to metal(loid) pollution. Currently, treatment and reuse of heavily contaminated dredged materials is not a cost-effective alternative to disposal landfill sites (Bert et al., 2009). In the Nord-Pas-de-Calais region (France), which is affected by intensive industrial activities, local authorities are required to manage contaminated landfill sites where large volumes of polluted dredged materials were deposited. The regional division of Voies Navigables de France (VNF) developed a management strategy for its disposal sites. This strategy includes the implementation of an environmental management system which aims to meet best practices and comply with environmental regulation in the field of human health and environment. The VNF is involved in the reclamation of its disposal sites into 'natural' and 'green' zones (Prevost, 2008). Metal immobilization in metal-contaminated sediments at some landfill sites may contribute to this environmental management strategy.

Metal immobilization aims at (1) changing speciation of trace elements (TE) in the substrate to reduce their soluble and exchangeable fractions, (2) limiting TE-uptake by plants, (3) reducing the direct exposure through soil by reducing metal availability to heterotrophic organisms, resulting in enhanced biodiversity (Vassilev et al., 2004). In situ immobilization of metal(loid)s is achieved by incorporating amendments into the soil, promoting their sorption and precipitation. In addition to immobilization effects, soil amendment can improve soil fertility by increasing pH, organic matter content, microbial activity, and moisture retention, and reducing soil compaction (Vangronsveld et al., 1995, 1996).

The efficiency of amendments is site-specific, depending on various factors among which pollutant types and soil properties (Kumpiene et al., 2008). Numerous soil amendments have been tested in pots, at pilot and field scale, for remediation purposes (Bes and Mench, 2008; Knox et al., 2001; Kumpiene et al., 2008). The effects of Thomas Basic Slag (TBS) and hydroxylapatite (HA) incorporation have only been investigated in batch and pot experiments (Bes and Mench, 2008; Boisson et al., 1999; Friesl et al., 2006; Mench et al., 1994a, 1994b; Mench et al., 2000; Misra and Chaturvedi, 2007; Negim et al., 2010; Panfili et al., 2005). Hydroxylapatite, a mineral from the phosphate group, is the major component of tooth enamel and bone mineral. It is mainly used for medical purposes. Hydroxyapatite powders can be synthesised via numerous production routes, using a range of different reactants. Some processing techniques include wet chemical methods (precipitation), hydrothermal techniques, hydrolysis of other calcium phosphates and sol-gel. Due to its particular properties including the sorption of metallic ions, HA can be useful for the management of contaminated groundwater and soil. The application of HA $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ to

contaminated soil immobilizes dissolved Pb (Ma et al., 1993). Other studies on metal stabilization by phosphorous compounds focused on reduction of plant uptake of metals, as well as reduction of their solubility and mobility (Knox et al., 2001). After HA incorporation into a contaminated soil, the concentrations of exchangeable metals decreased and plant uptake of these elements was reduced (Boisson et al., 1999). However, arsenic uptake by plants increased and there were nutrient deficiencies. Therefore it was concluded that HA application could be effective to immobilize Zn, Pb, Cu and Cd but was inappropriate in the case of mixed metal-arsenic pollution and when potential nutrient deficiencies may occur. Thomas Basic Slag (TBS) is an alkaline by-product of steel industry used as a fertilizer by farmers. It usually increases soil pH (Bes and Mench, 2008) and contains P, Mn, Mg, and Fe (Panfili et al., 2005). Soil treatment with TBS decreased Cd and Zn-soluble and $(\text{Ca}(\text{NO}_3)_2)$ exchangeable fractions and reduced Cd concentrations in tobacco shoots (Mench et al., 1994a, 1994b). Zinc availability decreased after TBS addition and this persisted over 5 months without any phytotoxicity (Mench et al., 2000). Bes and Mench (2008) studied the incorporation of 0.25% and 3.9% (DW) TBS into Cu-contaminated soils. Based on soil phytotoxicity, 3.9% TBS was one of the most efficient amendments even though high levels of Cu remained in the soil solution. Panfili et al. (2005) studied the effect of HA and TBS on Zn in a metal-contaminated sediment and suggested that the formation of Zn phosphate contributed to Zn immobilization.

Plants, bacteria and soil fauna, notably springtails (Collembola), can develop tolerant populations on metal-contaminated soils (Bes et al., 2010; Escarré et al., 2011; Gillet and Ponge, 2003; Lors et al., 2005; Ryan et al., 2004; Tyler et al., 1989; Vangronsveld et al., 1995, 1996). Vegetation reduces contaminant mobility by reducing

leaching, soil erosion, and improves the aesthetic value of formerly barren areas (Ruttens et al., 2006). The vegetation itself may contribute to metal immobilization in the rhizosphere and through the production of litter (Bouwman and Vangronsveld, 2004). When metal bioavailability and exposure decrease, due to vegetation or amendment or both, sensitive plants, bacteria and soil fauna can develop and participate in turn to the restoration of an ecosystem on contaminated soils (Bouwman et al., 2001; Lock et al., 2003; Lock and Janssen, 2003, 2005; Vangronsveld et al., 1995, 1996).

This field-scale work was performed at an experimental metal-contaminated sediment landfill site. The aim was to evaluate the efficiency of TBS and HA incorporation on metal(oid) immobilization. Efficient soil amendments may decrease metal(oid) extractability and shoot metal concentrations. In parallel, the potential adverse or beneficial effects of TBS and HA incorporation were investigated on an array of biological parameters related to plant and springtail communities composition and diversity, total and metal specific bacterial populations (anaerobic sulphate-reducing bacteria and aerobic sulphur-oxidising bacteria), springtail trophic status (gut contents), sediment ecotoxicity and sublethal effects in plants (maximum photochemical efficiency of photosystem II and antioxidant enzymes activity levels). This battery might be relevant to assess the efficiency of soil additives.

This is the first time that a full-scale study of metal immobilization combined with ecosystem monitoring is reported on a contaminated dredged sediment disposal site.

2. Materials and methods

2.1. Description of experimental site and sediment treatment

In May 2002, a field trial was set up in an agricultural area in Lallaing (North of France, 50°23'17''N and 3°11'59''E). Three plots of 60 m² and 40 cm depth were dug in an uncontaminated soil. These plots were filled with freshly dredged sediments from the nearby Scarpe canal (Pont de Râches). These sediments were contaminated due to past and present non-ferrous metal processing and smelting activities. Two plots were used to assess soil additives. Thomas Basic Slag (TBS) and a synthetic hydroxylapatite (HA), both in powdered forms, were singly incorporated into the sediment at a rate of 5% and 3% DW, respectively. TBS was obtained from Cedest[®] (Mâcon, France) and HA was from Brenntag[®] (Mülheim/Ruhr, Germany). The purity of HA was certified to 99% by the supplier. The third plot remained untreated (NT). After TBS and HA incorporation, the treated and untreated plots were mechanically homogenized with a crane shovel for two hours to ensure that the mixture was homogeneous. The plots were air dried for two months to reduce the sediment water content. The three plots were further subdivided into 9 sub-plots of 20 m² each, resulting in 3 untreated plots (NT), 3 HA-treated plots and 3 TBS-treated plots. Six of these plots were further sown with grasses (*Deschampsia cespitosa* and *Festuca rubra*) while the 3 remaining plots remained unplanted. This study reported the work performed on these unsown plots. From mid-spring 2003, spontaneous vegetation started to develop on these plots. Vegetation management (i.e. removal of selected species, harvest, fertilizer addition, and irrigation) was not carried out from this time to March 2007.

2.2. Collection of sediment and soil samples

In March 2007, top-sediments (0-20 cm depth) of the three tested plots (NT, HA, TBS) were sampled. Three surface sediment samples per plot were randomly collected with a hand auger to determine (pseudo)-total metal(oid) and extractable concentrations,

pH_{water} and sediment ecotoxicity. Six additional randomly collected samples were taken on each of the plots at a depth of about 20 cm using a hand auger for bacterial analyses (3 samples/plot) and a corer of 5 cm diameter for collembolan analyses (3 samples / plot). One composite sample per plot was formed with 5 sub-samples randomly collected to determine the other physico-chemical characteristics (particle size, organic carbon, total nitrogen, carbonates and cation exchange capacity). In parallel, an uncontaminated soil (0-20 cm depth), located outside the plots, was collected. This control area (CA) was selected because it had similar spontaneous vegetation to the plots. Physicochemical properties were determined on a composite sample made of 5 sub-samples collected below the vegetation of interest. Three additional soil samples were collected for collembolan analyses.

2.3. Sediment and soil characteristics

All samples were air dried until constant weight and sieved through 2 mm mesh size. All analyses on composites have been performed at the Laboratoire Départemental d'Analyses et de Recherche (LDAR), Laon, France using standard methods (particle size (NF X 31 – 107, 2003), organic carbon (NF ISO 14 235, oxidation method, 1998), total N (NF ISO 11261, colorimetric method, 1995), carbonates (NF ISO 10693, 2004), cation exchange capacity (CEC, NF X31-130, 1999).

A sample of 0.5 g of air-dried and sieved sediment and soil was weighed into 70 mL teflon microwave tubes .Eight mL of *aqua regia* containing 2 mL 67% concentrated HNO₃ and 6 mL 36% concentrated HCl (NF EN 13657, 2003) was added. Samples were heated in a microwave digester (MARS Xpress, CEM Corporation[®], Matthews, NC) to 180 °C for 20 min, with a 30 minute ramp time. After filtration through a 0.45 µm Whatman filter, the pseudo-total metal(oid) concentrations (As, Cd, Pb, Zn, Cu)

were determined using an Inductively Coupled Plasma Atomic Emission Spectrometer ICP-AES (Jobin Yvon[®], Longjumeau, France). To assess the analytical quality, a standard reference sediment material SRM 2704 (Buffalo River Sediment, Standard Reference Material) was subjected to the same protocol. Recoveries were: 100 % for Cd, 101% for Zn, 104% for Pb, 90% for Cu and 94% for As.

Soil pH was measured in 1:5 sediment/soil:water suspension using a glass electrode pH meter (NF ISO 10390, 1994).

Table 1 lists physico-chemical properties of contaminated sediments (NT, HA, TBS) and the uncontaminated soil (CA).

2.4. Extractable trace element analysis and sediment ecotoxicity

Extractable sediment metal(oid) concentrations of treated and untreated plots were determined after extraction with 0.01 M Ca(NO₃)₂. Prior to analysis, 20 g of air-dried and sieved sediment were shaken for 48 h with 40 mL of 0.01 M Ca(NO₃)₂ solution. Extracts were filtered through a 0.45 µm cellulose membrane and metal concentrations were measured using ICP-AES.

Ecotoxicity was assessed by two standardized bioassays: the bacterial Microtox[®] assay and the algal test, using *Vibrio fischeri* and *Pseudokirchneriella subcapitata* as test organisms, respectively. Those two assays were previously shown to be highly sensitive to leachates obtained from dredged materials (Piou et al., 2009). Prior to the bioassays, soil leachates were prepared according to the French standard AFNOR X31-210 (1991) and diluted (10 to 90%) with demineralised water. Ecotoxicity assays were run according to AFNOR T90-320 (1991) and ISO 8692 (1989) standards for Microtox[®] and algal bioassays, respectively. The half maximum effective concentration

(EC₅₀, Microtox[®] bioassay) and the 50% inhibitory concentration (IC₅₀, algal bioassay) were derived from dilution-effect curves. When the toxicity of leachates was not sufficient to reach 50% of effect, EC50% or IC₅₀ values were indeterminable and the % of effect observed at the 90% dilution was then reported.

2.5. Plant survey, biodiversity indices and plant analysis

2.5.1. Plant survey and similarity index

Vascular plants were surveyed in May and June 2007 in all plots using Lambinon et al. (2004) as a standard reference book for plant taxonomy. The total vegetation cover and the cover rate of mosses, herbaceous plants, shrubs and trees were assessed on each plot. Individuals per species and per plot were counted and numbers were related to plot area. Species richness corresponded to the number of species per plot.

A similarity index (Sørensen, 1948) was calculated to compare the composition of vegetation between (i) experimental plots (influence of treatments) and (ii) experimental plots and surrounding biotopes (identification of putative biotopes that may contribute to plot colonization).

2.5.2. Biodiversity indices

The diversity of vascular plants was assessed using the Shannon-Weaver index H' (Shannon and Weaver, 1949), calculated as

$$H' = - \sum_{i=1}^S p_i * \log_2 p_i$$

where p_i is the species proportion in a community composed of s species. The homogeneity of species distribution was assessed through the regularity index J of Piélou (1966).

2.5.3. Plant analyses

Calamagrostis epigejos, a grass, and *Urtica dioica*, a forb, were chosen for phytotoxicity and metal content analyses as both herb species were present in all plots and in the control area in sufficient abundance to sample 5 individuals per species of approximately similar size and sufficient biomass to perform analyses. Plant sampling occurred in June 2007.

2.5.3.1 Chlorophyll fluorescence

The fluorescence of chlorophyll a was measured on dark-adapted leaves of *C. epigejos* and *U. dioica* submitted to a saturating light pulse using a portable chlorophyll fluorimeter (Handy-PEA, Hansatech Instruments[®], Norfolk, UK). Basal fluorescence (F_0) and maximum fluorescence (F_m) values were used to derive F_v/F_m ratio (maximum photochemical efficiency of PSII), with the variable fluorescence $F_v = F_m - F_0$.

2.5.3.2 Antioxidant enzymes

Leaves from both plant species were collected from CA, NT, HA and TBS plots and immediately transferred to polypropylene tubes then frozen in liquid nitrogen. At the laboratory, leaf samples were crushed manually in a 125 mM phosphate buffer at pH 7.8 by using a porcelain mortar placed on ice. After centrifugation (15 000 g for 10 min at 4° C), the supernatant was used to determine antioxidant enzyme activities.

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GRD) activities were measured according to methods described by Dazy et al. (2008). The protein concentration was determined in plant extracts (Bradford, 1976); enzyme activity levels were expressed in $\mu\text{moles/min/mg}$ protein excepted for SOD for which one unit of activity corresponded to the amount of enzyme causing 50% inhibition of absorbance readings by comparison to control tubes lacking enzymes.

2.5.3.3 *Elemental concentrations in plant shoots*

Plant shoots were carefully washed (3 times) with distilled water and oven-dried at 40°C until constant weight. Weighed aliquots (0.5 g DW) were wet digested in 10 mL HNO_3 (65%) using a microwave digester (Mars Xpress) according to the following program: 3 min to reach 100 °C, 2 min to reach 140 °C, 2 min to reach 160 °C, 2 min to reach 180 °C and 20 min at 180 °C. Solutions were filtered to $<0.45 \mu\text{m}$ (Whatman filter) and concentrations of Cd, Zn, Pb, Cu, As, Ca, Fe, Mg, Mn, P, Na, K and Al were analyzed using ICP- AES (VARIAN 720 AES-ICP, Ulys[®], France). Blanks and certified reference material (trace elements in hay, IAEA V10, International Atomic Energy Agency, Vienna, Austria) were included for quality control. Recoveries were: 100% for Ca, 91% for Cu, 79% for Fe, 113% for Mg, 91% for As, 89% for Mn and Pb, 105% for K, 89% for Na, 118% for P, 87% for Zn, 93% for Cd and 67% for Al.

2.6 *Collembola identification and gut content observation*

After sampling at a depth of 20 cm, considering that the whole collembolan community was sampled on a surface of $\sim 20 \text{ cm}^2$, sediments (NT, HA, TBS) and soil

(CA) samples were embedded in polythene bags then transported to the laboratory. The extraction of arthropods started on the same day using the dry funnel (Berlese) method (Edwards and Fletcher, 1971) and was completed within 7-10 days. The daytime maximum temperature averaged 25-30 °C. Animals were collected and preserved in ethyl alcohol until sorting and identification. Sorting was done in alcohol under a dissecting microscope at x 20 magnification. All springtails were mounted in chloral-lactophenol then observed in a light microscope under phase contrast at x 400 magnification. Identification was done at the species level using keys by Bretfeld (1999), Fjellberg (1998), Gisin (1960), Hopkin (2000), Jordana et al. (1997), Potapov (2001) and Zimdars and Dunger (1994). Gut contents were observed by transparency and classified in 8 categories using morphological features observable in phase contrast light microscopy at x 400 magnification, and were counted according to the method devised by (Gillet and Ponge, 2003, 2005): algae, animal remains, bacteria, melanised and hyaline hyphae, fungal spores, holorganic humus, hemorganic humus, to which empty guts were added. The examination of gut contents may elucidate the composition of horizons in which the animals were living (Ponge, 2000). We sought to determine when toxicity forced the organisms to move to horizons in which they do not normally live or delete some food resources from their current diet (Gillet and Ponge, 2003).

2.7 Bacterial analyses

Sediment samples (NT, HA and TBS) were collected in polythene bags then transported to the laboratory. After homogenization, samples were maintained at 4°C before performing bacterial analyses. The enumeration of total sulphur-oxidising bacteria (SOB) was carried out on Nunc[®] Delta (Nalge Nunc International[®], Roskilde, Denmark) microplates of 96 wells (8 lines x 12 columns), on which each well contained

250 μL of specific medium. Nutrient Broth[®] (Difco[®], Detroit, USA) medium (diluted to 1/10), added to fungicidal cyclohexamide solution (0.2 g L^{-1}), was used for total bacterial count. The medium used to enumerate neutrophilic SOB contained 5 g $\text{Na}_2\text{SO}_3 \cdot 5\text{H}_2\text{O}$, 2 g K_2HPO_4 , 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g CaCl_2 , 0.002 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.005 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ and 3 ml bromocresol purple ($c = 0.02 \text{ g L}^{-1}$). The pH of the medium was initially adjusted to pH 6.5 with NaOH. For acidophilic SOB, the medium, which contained 1.5 g KH_2PO_4 , 0.1 g CaCl_2 , 0.1 g $\text{MgCl}_2 \cdot \text{H}_2\text{O}$, and 0.1 g NH_4Cl , was combined with 3 ml of bromocresol green ($c = 0.02 \text{ g L}^{-1}$). The pH was initially adjusted to 4.5 with HCl. A suspension containing 10 g of dry soil and 50 ml of Ringer solution was mixed in a Waring blender for 1.5 min at high speed. After decantation, the solution was successively diluted with Ringer solution to $10^{-1} - 10^{-8}$ (Ramsay, 1984). Each well was inoculated with 25 μL of solution. The wells of the eight lines corresponded to the eight tested dilutions. Each sample was inoculated in the wells of three columns and a column was not inoculated to control the sterility of the medium. As each microplate had twelve columns, only one microplate was used for the three samples of a given plot. The inoculated plates were incubated at 30°C for 15 days and then the coloured products were measured. Bacteria were counted using the Most Probable Numbers (MPN) method (De Man, 1977). Sulphate-reducing bacteria (SRB) were counted in tubes containing a solid medium specific to SRB (American Petroleum Institute, Washington, DC) (Lors et al., 2004).

2.8 Statistical analyses

A one-way ANOVA was used to detect treatment effects on metal concentrations, Fv/Fm ratio, antioxidant enzyme activities, species distribution, biodiversity indices, distribution of gut contents and bacterial numbers. When ANOVA

was significant, mean values were compared with post-hoc multiple-comparison procedures. When the sample weight was too low and variance homogeneity was not fulfilled, a non-parametric statistical test followed by a multiple-comparison procedure was performed. Correlation analysis, using Pearson product-moment correlation coefficient, was performed between metal concentrations and antioxidant enzymes activities in leaves. Statistica v7 (StatSoft Inc.) was used for bacterial calculations whereas XLSTAT[®] (Addinsoft[®], Paris, France) was used for springtail calculations. All other statistics were made with PASW statistics 18.

A semi-quantitative method was specially devised to make an overall assessment of beneficial or adverse effects of soil amendment (HA and TBS) addition on dredged sediments. For this purpose, the treatments (NT, HA, TBS) were ranked 1 to 3, a value of 1 being attributed to the treatment that maximized variables describing optimal features of plant, faunal and microbial communities (i.e. vegetation succession state, species richness, species diversity, equitability, abundance) and plant Fv/Fm ratio, or minimized those describing metal mobility, ecotoxicity, plant metal concentration and antioxidant enzyme activity,. Some variables cannot be taken into account in these calculations, either because they may respond to other factors than soil toxicity (acid vs neutral sulphur-oxidizing bacteria, collembolan gut contents, vegetation composition, pH), or because they do not differ significantly among treatments (plant Fv/Fm ratio for *C. epigejos*). Ranks were then averaged among the different variables which had been kept for the calculations. Overall differences between the three treatments NT, HA and TBS were then tested by a sign-test, using ranks as unit values.

3 Results

3.1. *Metal(oid) mobility and sediment ecotoxicity*

Extractable Pb and As concentrations in all plots were below quantification limits (0.07 and 0.1 mg kg⁻¹ DW for Pb and As, respectively). Cadmium and Zn showed a similar pattern with higher 0.01 M Ca(NO₃)₂-extractable concentrations in the NT plot than in the TBS-treated plot, while intermediate values were found in the HA-treated plot (Table 2). Expressed as percentages of the total metal concentrations, extractable Cd and Zn represented 0.14 and 0.07%, respectively, in the TBS-treated plot, whereas they represented 0.37 and 0.76% in the NT plot and 0.31 and 0.61% in the HA-treated plot. For Cu, no significant differences occurred across the plots.

Sediment leachates were moderately toxic to the bacteria *V. fischeri* since no EC₅₀ could be determined. Considering the % of effect measured at the 90% dilution, the ecotoxicity of sediment leachates could be ranked in the following order NT > HA > TBS (Table 2). Sediment leachates were more toxic to Algae, especially those prepared from NT and HA (IC₅₀ values being 12.8 and 19.2 %, respectively). TBS sediment leachate had only a slight inhibitory effect on Algae.

3.2. *Plant survey, plot similarity, source of colonization and element concentrations*

The composition of the plant community was influenced by the treatments of the plots as shown by plant survey and the number of individuals per species and per surface (Table 3). The TBS plot was mainly colonized by herbaceous plants while HA and NT plots were dominated by shrubs. The maximum height of shrubs was higher in HA plot than in NT plot. The TBS plot had the lowest plant species richness (n=14).

Based on Shannon-Weaver diversity and Pielou equitability indices, the HA plot had the highest plant diversity with a more homogeneous species distribution while TBS and NT plots showed a lower diversity and at least one species that dominated the overall community (Table 3).

In all plots, the dominant family was Poaceae with 5 species for HA, 4 species for TBS and 3 species for NT. Among Poaceae, only *C. epigejos* was present on all plots. *Calamagrostis epigejos* was more present on the NT plot (46.9%), than on TBS (30%) and HA plots (19.9%). On all plots, other plant species were abundant: *Carex hirta* (11.5%) and *Glechoma hederacea* (11.7%) on the NT plot, *Betula pendula* (12.7%) and *G. hederacea* (22.8%) on the HA plot, and *Phalaris arundinacea* (32.1%), *Galium aparine* (16.4%) and *Epilobium angustifolium* (11.7%) on the TBS plot. The second most abundant family was *Asteraceae* with three species. At least two *Asteraceae* were present on all plots. Perennial species dominated the three group communities. Plant species were native to the Nord-Pas-de-Calais region with no clear specificity linked to sediment traits except for some plants showing hygrophilic characteristics (e.g. *Eupatorium cannabinum*, *C. hirta*, *Carex cuprina*, *P. arundinacea*, *Salix alba*).

The Sørensen index, comparing vegetation composition between plots and between plots and surrounding biotopes, indicated that 85% of plant species were common between NT and HA plots whereas the TBS plot showed 60% and 57% species similarity with HA and NT plots, respectively (data not shown). The surrounding biotopes consisted of a slag heap, a ruderal zone, a canal bank, and an agricultural land. A similarity index indicated that the plant colonizers of the NT and HA plots mainly originated from the slag heap, with 45 and 41% of species similarity.

This result agreed with the direction of the south-easterly prevailing wind and the seed shadow colonization hypothesis (Clark et al., 2005), as the slag heap was situated south-east from the plots. The TBS plot showed less similarity with the slag heap (33%).

The concentrations of the chemical elements were measured in *C. epigejos* and *U. dioica* as both species were sampled in all plots (Table 4). Their shoot Pb and As concentrations were below quantification limits (1 and 0.5 mg kg⁻¹ for Pb and As, respectively). Shoot Cd and Zn concentrations for both species and Cu concentration for *U. dioica* were lower in CA than in the three treatments. The shoot Cd concentration was the lowest in *C. epigejos* grown on TBS whereas for *U. dioica* it did not differ across treatments. Shoot Zn concentration was the lowest in *C. epigejos* grown on TBS. The highest shoot P and K concentrations were measured in *U. dioica* grown on TBS. No other significant effect of TBS treatment on metal concentration was observed. The HA treatment did not decrease shoot Cd and Zn concentrations of *C. epigejos* and *U. dioica* compared to NT but increased shoot Cu in *C. epigejos*. Shoot Mg, Na, and Al concentrations in *C. epigejos* and shoot Fe concentration in *U. dioica* were higher in the HA plot than in the NP plot. The HA treatment did not influence other shoot element concentrations.

3.3. Antioxidant enzyme activities and chlorophyll fluorescence of leaves

Unstressed plants typically exhibit Fv/Fm ratios of 0.8-0.83 (Laposi et al., 2009), which was the case for *C. epigejos* on all plots (Fig. 1). For all antioxidant enzymes, lowest activities were determined in CA plants. In untreated sediment (NT), *C. epigejos* enzymes were significantly increased 1.2 to 3 fold depending on the enzyme considered.

For plants collected on HA plots, the activity of SOD, CAT, and GRD were similar to CA values, except APX which remained higher. For GPX, the HA treatment had no significant effect. This enzyme was less influenced than the others by the composition of the NT sediment since its activity only increased by a factor of 1.2 compared to plants harvested on CA plots. Plant enzymes responded differently to the TBS treatment. For SOD, CAT and APX, activities levels were not influenced since their values did not differ significantly from corresponding values in NT plants. GPX and GRD were both affected by TBS treatment, but in opposite ways, since GPX increased by comparison to NT values while GRD decreased.

Urtica dioica collected from CA and HA plots, and to a lesser extent NT plots, had all Fv/Fm ratios around 0.8 (Fig. 2). For TBS-plants, the Fv/Fm ratios were significantly lower. As noted for *C. epigejos*, for all antioxidant enzymes, the lowest activities were found for CA plants. For the untreated sediment (NT), *U. dioica* enzymes significantly increased by a factor 3 to 5. The HA and TBS treatments had opposite effects on antioxidant enzymes. All tested enzyme activities in *U. dioica* leaves decreased for HA compared to NT and tended to recover CA values. However, differences between HA and CA plants remained significant for SOD, APX and GPX activities. In contrast, TBS plants still exhibited high enzyme activities, similar to NT values (e.g. APX and CAT) or even higher (e.g. SOD, GPX, and GRD).

3.4. Correlations between shoot element concentrations and antioxidant enzyme activities

A correlation analysis was performed, between shoot element concentrations in *C. epigejos* and *U. dioica* and their antioxidant enzyme activities in leaves, to reveal significant linear relationships (Table 5). All enzyme activities negatively correlated

with shoot Cd concentration in *C. epigejos*. Manganese concentrations were positively correlated with 4 out of the 5 enzyme activities whereas Cu, Zn, Mg, and Na concentrations were correlated with GRD. For *U. dioica*, no relationship was found between shoot Cd concentration and any of the enzyme activities, but all enzyme activities were positively correlated with shoot Cu, Zn, P, and K concentrations.

3.5. *Collembolan diversity and gut contents*

A total of fourteen species were found in the four areas (Table 6). Neither total abundance nor biodiversity indices (species richness, Shannon index, equitability) showed significant differences between treatments. However, the TBS plot tended to harbour more specimens and species than NT and HA plots and CA. In the NT plot, there was no decrease in any of the four biodiversity indices when compared to CA, with a mean abundance of 20 specimens per core sample, shared between four species. At the species level, *Friesea truncata* was only present in the TBS samples.

The only significant effect on the gut contents of Collembola (Fig. 3) was an increase in the percent of specimens with hyaline fungal hyphae, which increased in all cores sampled in the HA plot, reaching an average of 16% compared to less than 3% for the other treatments. Hemorganic humus (amorphous organo-mineral matter) was dominant everywhere, indicating that the sediments (treated or not) were consumed by springtails, in particular by the dominant species *Mesaphorura florum*.

3.6. *Total and specific bacterial numbers*

The total bacterial population in all plots varied from 10^7 to 10^8 bacteria g^{-1} dry sediment, and was similar to that usually found in unpolluted topsoils (Robert, 1996;

Taylor et al., 2002) (Table 7). However, in the TBS plot, the total bacterial population (in 10^8 bacteria.g⁻¹ dry soil) was significantly higher (1.7) than in the NT plot (0.21).

In all plots, neutrophilic sulphur-oxidising bacteria (NSOB) were the most abundant group, with about 10^5 bacteria g⁻¹ dry sediment, whereas acidophilic sulphur-oxidising bacteria (ASOB) were the least abundant group with about 10^3 - 10^4 bacteria g⁻¹ dry sediment. The amendments did not modify the size of these bacterial populations.

The sulphate-reducing bacterial microflora (SRB) was about 10^1 - 10^2 bacteria g⁻¹ dry sediment and was of the same order of magnitude in all plots.

3.7. *Integration of data from the ecotoxicity battery*

Averaging ranks of all variables selected for the synthetic assessment of treatment effect (see Material and methods) indicated that TBS and HA exhibited similar favourable effects on the ecosystem when compared to the untreated sediment (NT) (Table 8). HA and TBS treatments ranked significantly above NT (one-sided t-tests, $P = 0.001$ and 0.011 , respectively) while they did not differ from each other (two-sided t-test, $P = 0.797$).

4. Discussion

Although the TBS application rate and the soil type used in this study differed from those investigated in other works, our results are broadly similar for Zn and Cd. The incorporation of TBS (5% DW) into the metal contaminated sediment decreased the 0.01 M Ca(NO₃)₂-extractable Cd and Zn fractions and shoot Cd and Zn concentrations of *C. Epigejos* (Tables 2 and 4), confirming previous findings (Mench et al., 1994a, 1994b, 2000). The TBS incorporation into a Cd-Zn-contaminated soil, reduced both the

Ca(NO₃)₂ extractability of Cd and Zn and their contents in tobacco shoots (Mench et al., 1994a, 1994b). In addition, TBS added into a Zn-contaminated soil increased soil pH, decreased extractable Zn and shoot Zn concentration in ryegrass, 3 and 5 months after soil treatment (Mench et al., 2000). Contrary to TBS, HA incorporation (3% DW) into the sediment did not decrease significantly Cd and Zn extractability nor Cd and Zn concentrations in plants (Tables 2 and 4). These results were not consistent with those of Boisson et al. (1999) who found a decrease of extractable Cd and Zn and metal concentrations in maize when compared to the untreated soil and with increasing HA application rate (0.5, 1 and 5% DW).

In contrast to other works (Boisson et al., 1999; Friesl et al., 2006), As-mobilization was not observed either in the presence of HA or TBS (Table 2). No amendment effect was recorded for Cu (Tables 2 and 4) whereas TBS incorporation (0.25 and 3.9% DW) into a Cu contaminated soil decreased Cu concentrations in the primary leaves of bean (Bes and Mench, 2008).

In our field study, TBS was the most efficient amendment to decrease Zn and Cd mobility and transfer to the plants. We hypothesise that Zn- and Cd-phosphates were precipitated, thereby immobilizing these elements in a non-available form as suggested by Panfili et al. (2005) who studied Zn speciation in the same metal-contaminated sediment treated with TBS. In our field experiment, the low efficiency of HA compared to TBS may be explained by the low solubility of HA at neutral pH (Table 1). As suggested by Miretzky and Fernandez-Cirelli (2007), the solubility of the phosphate phase is necessary for successful in situ treatment, metal immobilization by phosphorus being limited or inefficient when the matrix is neutral or alkaline.

As the essential nutrient concentrations did not decrease in plant shoots (Table 4), our results suggested that neither TBS nor HA led to deficiency problems in the tested plant species. In the presence of HA (5% DW), Boisson et al. (1999) reported a Mn-deficiency in maize. Neither treatment promoted the uptake of essential nutrients such as Ca except in the case of *U. dioica* where shoot P and K concentrations were increased by TBS addition. In primary leaves of bean, Ca concentration was enhanced in the presence of TBS whereas P concentration was not promoted (Bes and Mench, 2008; Negim et al., 2010).

In our study, sediment ecotoxicity was reduced by the incorporation of TBS and HA, TBS being the most efficient amendment.

At excessive concentrations in the soil solution, Cd, Cu, Pb and Zn exert toxic effects on plants, including alterations in photosynthetic and respiration processes or inhibition of plant growth. They may also stimulate the formation of reactive oxygen species (Dazy et al., 2009). To prevent oxidative damage, plant cells usually use enzymatic protection mechanisms, such as superoxide dismutase, catalase, and peroxidases, and metabolites such as ascorbate and glutathione (Dazy et al., 2008). Following amendment of the soil, a decrease in phytotoxicity and a recovery in plant antioxidant defence to control values are expected as a result of metal-immobilization processes (Ruttens et al., 2006; Vangronsveld et al., 1995, 1996). In the NT plot, all antioxidant enzyme activities of both plant species were increased compared to the CA plot, indicating that the plants suffered from oxidative stress. The increase in shoot Zn and Cu concentrations were consistent with enzymatic activity increases, suggesting that these metals accounted for the stress response recorded in plants growing on untreated dredged sediment. This was not the case for Cd, as no correlation for *U.*

dioica and no positive correlation for *C. epigejos* were found. The addition of HA permitted an almost total recovery of antioxidant enzyme activities to CA values, whereas the addition of TBS did not. Thus, HA was a better amendment for keeping the stress responses of plants at a low level. Levels of chlorophyll fluorescence (Fig. 1 and Fig. 2) indicated that the plants were not stressed on NT, HA and TBS plots. There are few studies that report the effect of TBS on phytotoxicity and an absence of studies on the effect of HA on phytotoxicity. After the incorporation of TBS (0.25% and 3.9%) into a Cu-contaminated soil, the chlorophyll density of primary leaves of bean decreased and guaiacol-peroxidase activity in roots fell to control level (Bes and Mench, 2008). In another experiment, TBS added to a Zn-contaminated soil restored guaiacol-peroxidase activity of the primary leaves in beans to control values (Mench et al., 2000).

Most of the plant species present on the NT plot were also observed on the surrounding uncontaminated biotopes (slag heap and ruderal zone), which were similarly firstly devoid of vegetation. Thus, colonization of the NT plot was not limited by the total metal concentration in the sediment, which is consistent with previous findings (Escarré et al., 2011). Madejon et al. (2006) showed that the application of amendments to a metal contaminated soil improved spontaneous colonization and establishment of early-successional plants. In our study, all plots were covered by dense vegetation (close to 100%) but differed in plant community composition and biodiversity descriptors. This may indicate that the amendment application was not a key factor for colonization and establishment of spontaneous vegetation but an important factor that could modify vegetation composition and dynamics. The addition of TBS or HA to the sediment influenced the dynamic process of plant colonization, the three plots showing differences in composition of the plant community. The large

increase in pH following addition of TBS to the sediment (7.4 to 10.2 in May 2002), compared to the modest increase (7.4 to 7.7 in May 2002) in the presence of HA, may be responsible for the main changes in plant community in the TBS plot compared to other plots. Only spontaneous vegetation that is able to tolerate alkaline pHs could colonize this plot. Elevated pH favours the establishment of herbaceous rather than woody species (Skousen et al., 1994). Five years after the single application, the pH in the TBS-treated sediment decreased to 7.7 while pHs of NT and HA plots were 7.0 and 7.3, respectively (Table 1). This decrease may be sufficient to favour the establishment of *B. pendula* in the next few years, as two seedlings were noticed in the TBS plot. *Calamagrostis epigejos* is known as a competitive social grass able to arrest plant succession by forming extended dense areas (Prach, 2003). At maturity, it can reach 1.5 m in height thereby shading smaller or slowly-growing plant species (Rebele and Lehmann, 2001). As the higher rate of *C. epigejos* was recorded on the TBS plot, this plant would contribute to the maintenance of early stage succession vegetation on this plot.

The highest level of plant species richness was found in the presence of HA (26 species out of 16 families) compared to the NT plot (21 species out of 14 families). In contrast, TBS did not favour species richness as it accounted for the least value (14 species out of 9 families). This is paradoxical since the TBS plot had the lowest concentrations of extractable Zn and Cd and the lowest sediment ecotoxicity, but the competitive exclusion of plants by *C. epigejos* possibly outweighed effects of decreased toxicity.

Concerning diversity indices, our H' values were much higher than those given by Conesa et al. (2007) and Dazy et al. (2008) who studied plant communities growing

respectively on Zn-Pb-contaminated tailings and experimental plots filled with a multicontaminated soil. However, such comparisons of H' values may be confounded by different soil and pollution parameters as well as the time elapsed after colonization.. For Dazy et al. (2008), the survey was performed after 14 months of colonization whereas in this study the survey was done 5 years after colonization.

The influence of metal contamination on natural springtail communities has been documented (Bengtsson and Rundgren, 1988; Chauvat and Ponge, 2002; Gillet and Ponge, 2002, 2003; Lock et al. 2003; Strojan, 1978). In these works, no major adverse effects of metal contamination were recorded. Springtails were abundant even in highly contaminated sites, which indicates the resistance of this group to toxic effect of metals and other environmental stresses (Hopkin, 1997), exemplified by high EC₅₀ values in *Folsomia candida* reproduction test at 20 °C for Cd (590 µg.g⁻¹), Cu (700 µg.g⁻¹), Zn (900 µg.g⁻¹) and Pb (2790 µg.g⁻¹) (Sandifer and Hopkin, 1997). In our study, neither total abundance nor biodiversity indices showed significant differences between NT plot and CA, showing the normal colonization by springtails of the NT plot. The addition of TBS and HA did not change the abundance or biodiversity indices, showing no effect of these amendments on the springtail community. Some effect may have been expected on the TBS plot as Lock et al. (2003) reported a significant negative relationship between the metal extractable fraction (0.01 M CaCl₂ solution) and the number of springtail species. In our study, the gut content analyses showed that untreated and amended dredged sediments were consumed by springtails (Fig. 3).

In soils that are highly contaminated with metal(loid)s, a lower number of bacteria than in uncontaminated soil could be expected, as a result of the harmful effects of the contaminants on soil bacterial activity, especially bacterial respiration and soil

bacterial biomass (Renella et al., 2005; Sobolev and Begonia, 2008). In our study, the total bacterial population in the NT plot was similar to that usually found in unpolluted topsoils (Taylor et al., 2002).

The addition of TBS to the soil improved the total bacterial population, probably as a consequence of the low Cd-Zn-extractable fraction on this plot.

Bacteria play an important role in increasing or decreasing metal availability, notably in sediments (Bert et al., 2009). Bioleaching of metals is caused by sulphur-oxidising bacteria (SOB) whereas anaerobic sulphate-reducing bacteria (SRB) can precipitate metals. Neutrophilic and acidophilic SOB were found in similar numbers in all plots, showing no amendment effect. In all sediment plots, neutrophilic SOB were more numerous than acidophilic SOB, due to soil pH close to neutrality (Table 1). The moderate development of acidophilic SOB was consistent with the results of Lors et al. (2004) who reported an acidophilic sulphur-oxidising population of about 10^4 bacteria g^{-1} dry soil in a similar sediment. These results indicated that metals might be released in the case of metal sulphide availability.

Sulphate-reducing bacteria were present in the same low range in the three sediment plots, showing no amendment effect (Table 7). The low amount of SRB may be explained by the sampling performed on the first 20 cm depth layer corresponding to aerobic conditions, not favourable to the growth of SRB that are strictly anaerobic. However, during wet periods, the saturation of the soil pore system might induce anoxic conditions that would promote the size of the SRB community, leading to increased metal sulphide content.

5. Conclusions

In this paper, TBS and HA were investigated in an experimental metal-contaminated sediment landfill site to evaluate their effects on metal extractability, sediment ecotoxicity and on living organisms such as plants, bacteria and springtails.

The incorporation of TBS into the metal contaminated sediment decreased the extractable fraction of Cd and Zn. In contrast, HA did not decrease Cd and Zn extractability. TBS at a rate of 5% DW was effective in Cd and Zn immobilization, did not cause any nutrient imbalance or sediment ecotoxicity, and improved the bacterial activity.

However, TBS application had either no influence or a negative effect, on maximum photochemical efficiency of PSII and antioxidant enzymes in plants. The bioavailability of the compound(s) responsible for those biological responses was not decreased by TBS treatment. Even if plants successfully colonized the TBS plot, they remained under stress.

Our study revealed (i) the favourable influence of both HA and TBS compared to NT, (ii) the better influence of HA on vegetation development and physiological welfare (as measured by PSII measurements and antioxidant enzymes), (iii) the better influence of TBS on sediment ecotoxicity, metal immobilization, microbial population and Cd-Zn accumulation in *C. epigejos*. The choice of using metal immobilization with TBS rather than HA as a tool in the management of dredged sediment landfill sites will depend on the objectives of such strategy, i.e. to limit metal transfer and sediment ecotoxicity or to favour plant diversity and welfare. Realising both objectives requires a study of a mixture of both soil amendments.

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886

Figure captions

Figure 1.

Fv/Fm ratios and antioxidant enzyme activities ($\mu\text{moles}/\text{min}/\text{mg}$ proteins excepted for SOD expressed as U/mg proteins) in *C. epigejos* leaves collected from control area (CA), untreated plot (NT), plots treated with hydroxylapatite (HA) or Thomas basic slag (TBS). Different letters indicate significant differences between plots according to post-hoc Tukey HSD tests run when ANOVA was significant.

Figure 2.

Fv/Fm ratios and antioxidant enzyme activities ($\mu\text{moles}/\text{min}/\text{mg}$ proteins excepted for SOD expressed as U/mg proteins) in *U. dioica* leaves collected from control area (CA), untreated plot (NT), plots treated with hydroxylapatite (HA) or Thomas basic slag (TBS). Different letters indicate significant differences between plots according to post-hoc Tukey HSD tests run when ANOVA was significant.

Figure 3.

Distribution of gut contents among the 403 collembolan specimens collected in three treatments and in the control area (CA). Letters indicate significant differences among means at 0.05 level (ANOVA followed by SNK procedure).

Table 1

Main characteristics and (pseudo)-total metal concentrations of sediments (NT, untreated; HA, hydroxylapatite; TBS, Thomas Basic Slag) and the control area (CA). For trace elements (except for CA) and pH, values are means of triplicate measurements and their standard deviation.

	CA	NT	HA	TBS
Particle size distribution (%)				
Sand	13.2	69.6	60.1	65.4
Silt	70.3	14	21.1	19.1
Clay	16.5	16.4	18.8	15.5
Organic C (%)	1.64	3.2	4.1	3.7
N (g kg⁻¹)	1.6	1.62	1.67	2.04
CaCO₃ (g kg⁻¹)	63	76	76	105
CEC (cmol kg⁻¹)	13.3	7.2	8.3	11.3
pH_{water}	7.6 ± 0.1	7.0 ± 0,1	7.3 ± 0,1	7.7 ± 0,3
Zn (mg kg⁻¹ DW)	75	3250 ± 319	2521 ± 461	3029 ± 217
Cd (mg kg⁻¹ DW)	0.4	84 ± 2	67 ± 11	86 ± 8
Pb (mg kg⁻¹ DW)	27	448 ± 82	389 ± 96	413 ± 57
Cu (mg kg⁻¹ DW)	17	93 ± 13	94 ± 11	123 ± 29
As (mg kg⁻¹ DW)	8	53 ± 4	48 ± 4	45 ± 2

Table 2

Concentrations of trace elements extracted from soils by 0.01 M Ca(NO₃)₂ (means of triplicate values ± standard deviation) and corresponding leachate ecotoxicity. Significant differences among means at 0.05 level were indicated by different letters. nr = not reached

	NT	HA	TBS
Zn (mg kg⁻¹ DW)	12.05a ± 0.96	7.77ab ± 0.33	2.07b ± 2.73
Cd	0.64a ± 0.12	0.41ab ± 0.04	0.12b ± 0.13
Pb	<0.07	<0.07	<0.07
Cu	0.12a ± 0.01	0.13a ± 0.02	0.21a ± 0.14
As	<0.1	<0.1	<0.1
Microtox EC₅₀ (%)	nr	nr	nr
Microtox	44	20.5	6.3
(% luminescent inhibition at 90%)			
Algae test IC₅₀ (%)	12.8	19.2	nr
Algal test	100	100	36.4
(% inhibition at 90%)			

918 **Table 3**

919 Composition of the plant community in the three treatment plots. Species richness is the
 920 number of species. H' is the Shannon-Weaver index. J is the Pielou equitability index.

Family	Species	Plant type	Plant life cycle	NT	HA	TBS
Apiaceae	<i>Pastinaca sativa</i>	Herbaceous	Annual	0.47	0.46	0
Asteraceae	<i>Eupatorium cannabinum</i>	Herbaceous	Perennial	0	3.15	0.29
	<i>Achillea millefolium</i>	Herbaceous	Annual	0.12	7.59	0
	<i>Cirsium arvense</i>	Herbaceous	Perennial	0.47	0	1.39
Brassicaceae	<i>Cardamine hirsuta</i>	Herbaceous	Annual	6.18	1.76	0.19
Boraginaceae	<i>Myosotis arvense</i>	Herbaceous	Annual	0.12	0.09	0
Convolvulaceae	<i>Convolvulus arvensis</i>	Herbaceous	Perennial	0	0.46	0
Cyperaceae	<i>Carex hirta</i>	Herbaceous	Perennial	11.55	1.85	0.33
	<i>Carex cuprina</i>	Herbaceous	Perennial	0.12	0.09	0
Lamiaceae	<i>Glechoma hederacea</i>	Herbaceous	Perennial	11.67	22.85	7.08
	<i>Lycopus europaeus</i>	Herbaceous	Perennial	0.70	1.57	0
Onagraceae	<i>Epilobium angustifolium</i>	Herbaceous	Annual	0.35	1.2	11.76
Poaceae	<i>Deschampsia cespitosa</i>	Herbaceous	Perennial	1.52	1.76	0
	<i>Festuca rubra</i>	Herbaceous	Perennial	3.73	6.85	0
	<i>Phalaris arundinacea</i>	Herbaceous	Perennial	0	0.93	33.09

	<i>Calamagrostis epigejos</i>	Herbaceous	Perennial	46.91	19.98	30.00
	<i>Holcus lanatus</i>	Herbaceous	Perennial	0	2.22	0.1
	<i>Poa trivialis</i>	Herbaceous	Perennial	0	0	0.14
Ranunculaceae	<i>Ranunculus repens</i>	Herbaceous	Perennial	0.12	1.11	0
Rubiaceae	<i>Galium aparine</i>	Herbaceous	Annual	2.22	2.13	16.40
Scrophulariaceae	<i>Linaria vulgaris</i>	Herbaceous	Perennial	0	1.67	0
Urticaceae	<i>Urtica dioica</i>	Herbaceous	Perennial	2.33	4.63	0.86
Betulaceae	<i>Betula pendula</i>	Tree	Perennial	8.05	12.67	0.05
	<i>Betula pubescens</i>	Tree	Perennial	1.87	2.31	0.05
Fagaceae	<i>Quercus robur</i>	Tree	Perennial	0.12	0.28	0
Salicaceae	<i>Salix sp.</i>	Tree	Perennial	0	0.46	0
	<i>Salix alba</i>	Tree	Perennial	0.12	1.02	0
	<i>Salix capraea</i>	Tree	Perennial	1.40	0.93	0

Species richness	21	26	14
H'	2.7	3.6	2.35
J	0.62	0.77	0.62

921 For species, values were percent numbers of individuals per species and per unit
922 surface.

923

924 **Table 4**925 Concentrations (mg/kg DW) in shoots of *Calamagrostis epigejos* and *Urtica dioica*.

	CA	NT	HA	TBS
<i>Calamagrostis epigejos</i>				
Cd	< 0.05	2.16 (a) ± 1.4	2.51 (a) ± 1.1	0.34 (b) ± 0.18
Zn	23 (a) ± 9	121 (c) ± 58	98 (bc) ± 32.6	44 (ab) ± 20
Pb	<1	<1	<1	<1
Cu	4.5 (ab) ± 0.9	3.5 (a) ± 0.3	5.1 (b) ± 0.7	4.3 (ab) ± 0.2
As	< 0.5	< 0.5	< 0.5	< 0.5
Ca	405 ± 331	617 ± 115	769 ± 273	791 ± 243
Fe	23 ± 2	52 ± 56	33 ± 5	23 ± 1
Mg	453 (b) ± 121	251 (a) ± 83	532 (b) ± 147	446 (ab) ± 62
Mn	16 (a) ± 5	51 (b) ± 8	13 (a) ± 3	15 (a) ± 1
P	1570 ± 247	1530 ± 189	1863 ± 875	1175 ± 235
Na	123 (ab) ± 4	115 (a) ± 4	131 (b) ± 12	119 (ab) ± 13
K	12407 (a) ± 1329	14523 (ab) ± 1543	16648 (b) ± 2620	13074 (a) ± 1288
Al	n.d	5 (a) ± 2	8.5 (b) ± 1.6	24 (c) ± 1
<i>Urtica dioica</i>				
Cd	0.06 (a) ± 0.01	1.5 (b) ± 1.3	0.9 (ab) ± 0.3	0.36 (ab) ± 0.18
Zn	12 (a) ± 1	210 (b) ± 104	184 (b) ± 28	145 (b) ± 21
Pb	<1	<1	<1	<1
Cu	4.0 (a) ± 0.5	6.9 (b) ± 1.9	6.8 (b) ± 1.9	7.4 (b) ± 0.9
As	<0.5	<0.5	<0.5	<0.5
Ca	19719 ± 8227	20945 ± 3292	24398 ± 2407	23901 ± 3334
Fe	35 (a) ± 6	51 (ab) ± 10	60 (b) ± 12	43 (ab) ± 11
Mg	1726 ± 1753	1278 ± 433	2176 ± 285	1722 ± 241
Mn	13 ± 3	12 ± 6	11 ± 2	12 ± 2
P	2314 (a) ± 267	4229 (b) ± 465	4310 (b) ± 841	6096 (c) ± 563
Na	136 ± 38	177 ± 41	162 ± 20	149 ± 12
K	17355 (a) ± 4593	19029 (ab) ± 1434	23699 (bc) ± 1721	27963 (c) ± 2117
Al	n.d	20 ± 8	32 ± 10	28 ± 12

926 Mean values and standard deviations (n=5; mg kg⁻¹ DW). Different letters indicate
 927 significant differences among plots for α=0.05.

928

Table 5. Correlation analysis between shoot element concentrations and antioxidant enzyme activities in leaves (n=20).

<i>C. epigejos</i>					
	SOD	CAT	APX	GPX	GRD
Cd	-0.68**	-0.67**	-0.79***	-0.61**	-0.58**
Zn	0.31ns	0.35ns	0.56**	0.01ns	0.52*
Cu	-0.27ns	-0.48*	-0.38ns	0.00ns	-0.56*
Mn	0.55*	0.60**	0.53*	-0.11ns	0.80***
P	0.09ns	-0.02ns	-0.18ns	0.13ns	-0.23ns
Ca	0.37ns	0.18ns	0.38ns	0.44ns	0.08ns
K	0.06ns	-0.01ns	0.22ns	-0.03ns	0.14ns
Mg	-0.30ns	-0.43ns	-0.52*	-0.32ns	-0.56*
Na	-0.33ns	-0.54*	-0.19ns	-0.21ns	-0.50*
<i>U. dioica</i>					
	SOD	CAT	APX	GPX	GRD
Cd	0.22ns	0.24ns	0.30ns	0.32ns	0.11ns
Zn	0.48*	0.47*	0.56**	0.61**	0.45*
Cu	0.51*	0.49*	0.56*	0.60**	0.70**
Mn	-0.07ns	-0.02ns	-0.08ns	-0.04ns	0.08ns
P	0.79***	0.74***	0.79***	0.85***	0.82***
Ca	0.10ns	0.12ns	0.18ns	0.21ns	0.26ns
K	0.54*	0.45*	0.48*	0.56*	0.60**
Mg	-0.20ns	-0.21ns	-0.10ns	-0.07ns	-0.11ns
Na	0.22ns	0.24ns	0.28ns	0.22ns	0.02ns

ns: not significant, ***, **, *, significant at a probability level $P < 0.001, 0.01, 0.05$, respectively.

Table 6. Species distribution and biodiversity indices of collembolan species.

ANOVA: NS = not significant; ** P < 0.01. Post-hoc tests (SNK procedure):

significant differences indicated by different letters

	CA	NT	HA	TBS	F value
<i>Dicyrtomina minuta</i>	0	0	0	0.33±0.27	1 NS
<i>Entomobrya sp.</i>	0	1.33±1.09	0	0	1 NS
<i>Folsomia candida</i>	0.33±0.27	0.33±0.27	0	1±0.82	0.58 NS
<i>Friesea truncata</i>	0 ^b	0 ^b	0 ^b	4±1.25 ^a	6.86**
<i>Hererosminthurus claviger</i>	0.33±0.27	0	0	0	1 NS
<i>Isotomodes productus</i>	0.67±0.54	0	0	2.67±1.78	1.22 NS
<i>Lepidocyrtus lignorum</i>	0	0	0	0.33±0.27	1 NS
<i>Mesaphorura florum</i>	1.33±0.72	6.33±2.13	21.67±7.92	25.33±8.57	2.56 NS
<i>Mesaphorura macrochaeta</i>	3.33±2.72	0	0.67±0.27	0.67±0.54	0.75 NS
<i>Mesaphorura pongei</i>	0	0	0	1.33±1.09	1 NS
<i>Orchesella sp.</i>	0	0.33±0.27	0.33±0.27	0	0.67 NS
<i>Parisotoma notabilis</i>	6.67±1.96	4.67±1.66	5.67±2.13	0.33±0.27	1.85 NS
<i>Proisotoma minima</i>	7±2.45	9±2.94	0.67±0.54	7±2.36	1.71 NS
<i>Tomocerus vulgaris</i>	0	0	0.33±0.27	0	1 NS
Total abundance	19.67±3.07	22±5.44	29.33±6.4	43±10.6	1.53 NS
Species richness	4±0.47	4	3.67±0.27	5.67±1.19	1.28 NS
Shannon Index	1.44±0.21	1.65±0.07	1.09±0.32	1.78±0.26	1.09 NS
Equitability Index	0.72±0.04	0.82±0.04	0.56±0.15	0.75±0.03	1.25 NS

943

944

Table 7. Total and specific bacterial numbers

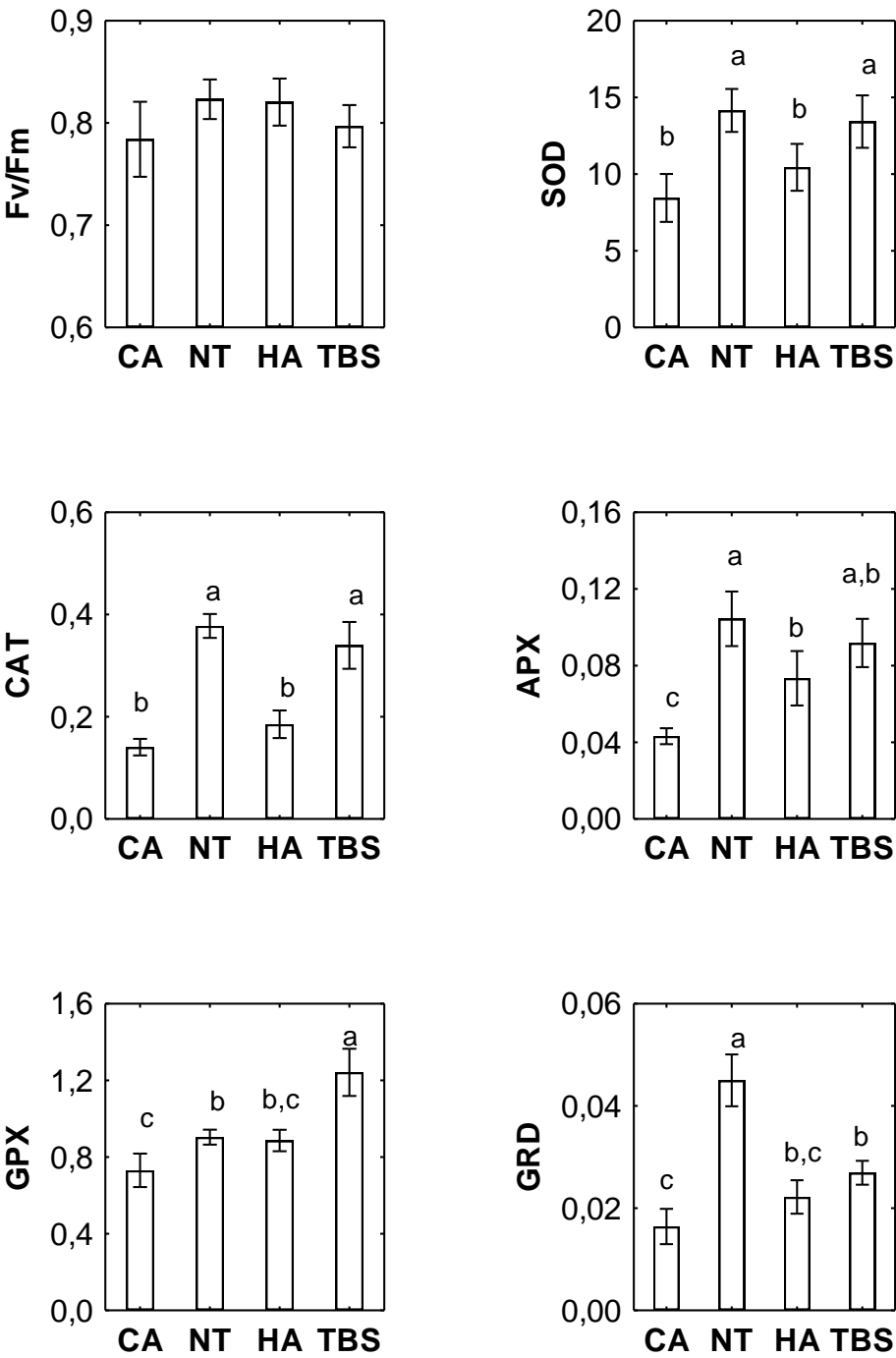
Counts of total bacteria, acidophilic sulphur-oxidising bacteria (ASOB), neutrophilic sulphur-oxidising bacteria (NSOB) and sulphate-reducing bacteria (SRB) (expressed as bacteria g^{-1} dry soil) collected from untreated plot (NT), plot treated with hydroxylapatite (HA) or Thomas basic slag (TBS). Values are expressed as means \pm standard deviation in triplicate samples. Values with the same letters are not significantly different according to ANOVA used Newman-Keuls (SNK) test at $P \leq 0.05$.

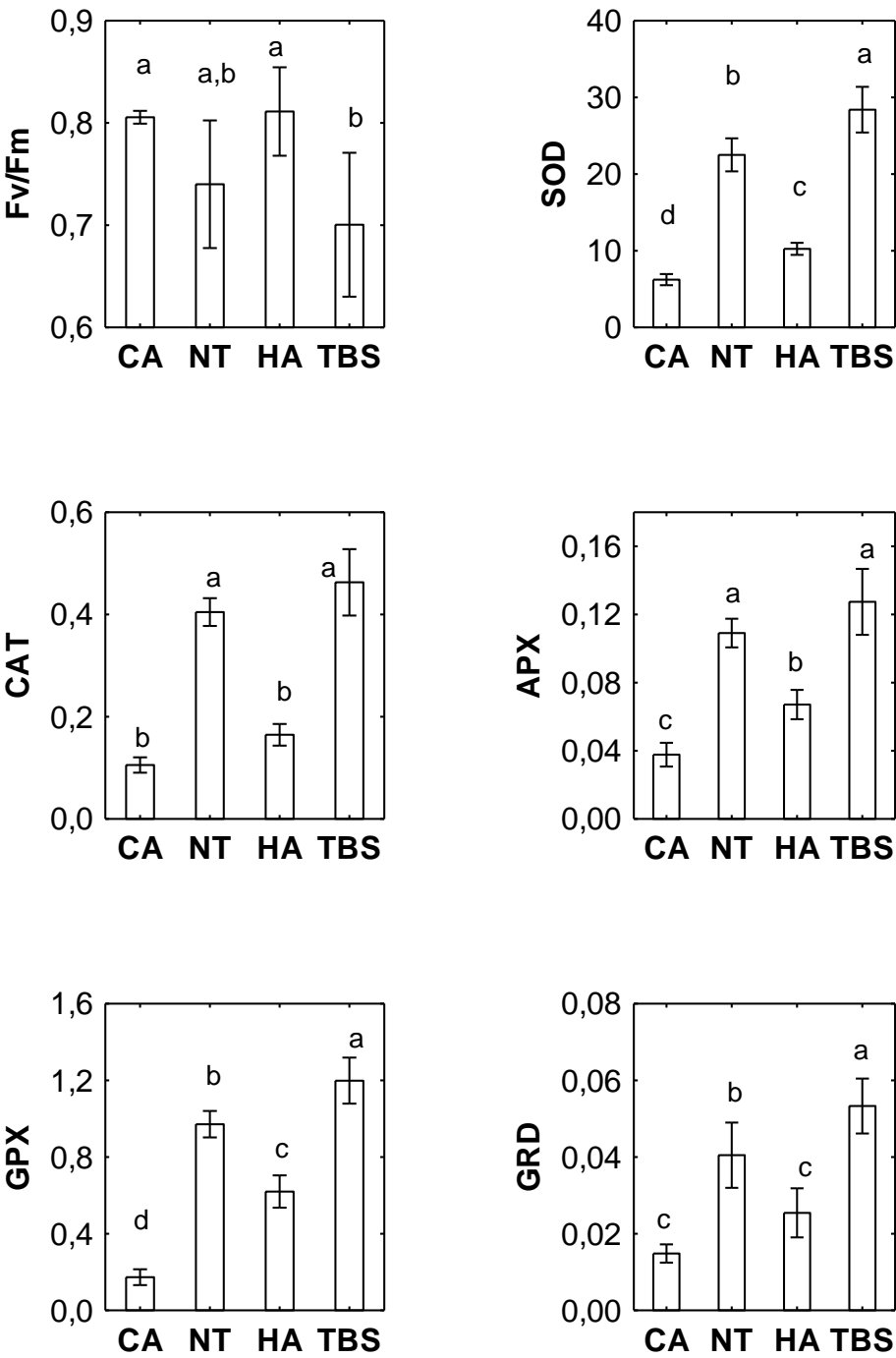
	NT	HA	TBS
Total bacteria	$2.12 \cdot 10^7$ $\pm 5.09 \cdot 10^5$ c	$5.58 \cdot 10^7$ $\pm 7.90 \cdot 10^6$ b	$1.75 \cdot 10^8$ $\pm 1.86 \cdot 10^6$ a
ASOB	$3.69 \cdot 10^3$ $\pm 2.31 \cdot 10^1$ a	$7.36 \cdot 10^3$ $\pm 3.19 \cdot 10^3$ a	$2.93 \cdot 10^3$ $\pm 1.29 \cdot 10^3$ a
NSOB	$1.00 \cdot 10^5$ $\pm 6.78 \cdot 10^4$ a	$3.05 \cdot 10^5$ $\pm 1.15 \cdot 10^5$ a	$1.45 \cdot 10^5$ $\pm 4.62 \cdot 10^4$ a
SRB	$6.97 \cdot 10^1$ $\pm 4.04 \cdot 10^1$ a	$5.97 \cdot 10^1$ $\pm 2.89 \cdot 10^1$ a	$5.97 \cdot 10^1$ $\pm 2.89 \cdot 10^1$ a

Table 8

Treatments (NT = Untreated, HA = Hydroxyapatite, TBS = Thomas Basic Slag) ranked according to 24 variables describing optimal features of plant, faunal and microbial communities and sediment toxicity. Average ranks are indicated by mean \pm standard deviation.

Variable	NT	HA	TBS
Plant, faunal and microbial communities good health			
Vegetation succession state	2	1	3
Plant species richness	2	1	3
Plant species diversity	2	1	3
Plant species equitability	2.5	1	2.5
Fv/Fm in <i>U. dioica</i>	2	1	3
Collembola abundance	3	2	1
Collembola species richness	2	3	1
Collembola species diversity	2	3	1
Collembola species equitability	1	3	2
Total bacteria abundance	3	2	1
Sediment toxicity			
Cd mobility	3	2	1
Zn mobility	3	2	1
Microtox	3	2	1
Algal test	2.5	2.5	1
Cd in <i>C. epigejos</i>	2	3	1
Zn in <i>C. epigejos</i>	3	2	1
Cd in <i>Urtica</i>	3	2	1
Zn in <i>Urtica</i>	3	2	1
SOD in <i>C. epigejos</i>	3	1	2
CAT in <i>C. epigejos</i>	3	1	2
APX in <i>C. epigejos</i>	3	1	2
SOD in <i>U. dioica</i>	2	1	3
CAT in <i>U. dioica</i>	2	1	3
APX in <i>U. dioica</i>	2	1	3
Average rank	2.46\pm0.57	1.73\pm0.76	1.81\pm0.9





969 **FIGURE 3**

